Fumagillin Reduces Adipose Tissue Formation in Murine Models of Nutritionally Induced Obesity

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The effect of fumagillin (a methionine aminopeptidase-type 2 (Met-AP2) inhibitor, with antiangiogenic properties) was investigated in murine models of diet-induced obesity. Eleven-week-old male C57Bl/6 mice (group 1) were given fumagillin by oral gavage at a dose of 1 mg/kg/day during 4 weeks while fed a high-fat diet (HFD) (20.1 kJ/g), and control mice (group 2) received solvent and were pair-fed. At the end of the experiment, body weights in group 1 were significantly lower as compared to group 2 ($P < 0.0005$). The subcutaneous (SC) and gonadal (GON) fat mass was also significantly lower in group 1 ($P < 0.005$ and $P < 0.05$, respectively). Adipocytes were smaller in adipose tissues of mice in group 1, associated with higher adipocyte density. Blood vessel density normalized to adipocyte density was lower in group 1 adipose tissues. However, in mice with established obesity monitored to maintain the same body weight and fat mass as controls, short-term fumagillin administration was also associated with adipocyte hypotrophy ($P = 0.01$) without affecting blood vessel size or density. Thus, treatment with fumagillin impaired diet-induced obesity in mice, associated with adipocyte hypotrophy but without marked effect on adipose tissue angiogenesis.

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INTRODUCTION

Over the past decades, obesity and its consequences worldwide have become a major health problem (1,2). Adipose tissue, unlike other organs, grows and develops continuously throughout life. To supply growing adipose tissues with nutrients and oxygen, the vasculature responds by increasing the number and/or size of blood vessels. In early stage development of adipose tissue, adipogenesis is tightly associated with angiogenesis (3). It was shown that exposure of mice to cold led to activation of angiogenesis in white and brown adipose tissues, associated with upregulation of proangiogenic factors and downregulation of endogenous angiogenesis inhibitors (4). Pro- and antiangiogenic compounds occurring in adipose tissue have been reviewed elsewhere (5). Several previous studies have suggested the potential to reduce adipose tissue development by inhitibion of angiogenesis (6–9). A promising approach may be the use of methionine aminopeptidase-type 2 (Met-AP2) inhibitors, such as the fumagillin-like synthetic compound TNP-470 (AGM-1470) (6,8).

Met-AP2 is one of two known metalloenzymes that cotranslationally remove the N-terminal methionine from nascent proteins. Met-AP2 nonenzymatically inhibits phosphorylation of eukaryotic initiation factor-2a by which it positively regulates translation (10). At the cellular level, blocking Met-AP2 inhibits the growth of endothelial cells in a p53 and p21-dependent process. Met-AP2 inhibitors block progression of the cell cycle into S-phase, causing arrest in late G, by activating p53 and causing accumulation of p21. They also decrease the expression of proliferating cell nuclear antigen (11,12). Fumagillin is a natural product isolated from *Aspergillus fumigatus* in 1949, whereas its molecular target, Met-AP2, was only identified in 1997 (10). It acts by covalently modifying His231 in the active site of Met-AP2 and does not inhibit Met-AP1 (10,13). In early clinical studies, it was tested primarily for oncologic indications and as an antiparasitic agent; it was well tolerated and associated with few adverse effects (14–16).

In this study, we have investigated the effect of fumagillin on adipose tissue development in murine models of nutritionally obesity.

METHODS AND PROCEDURES

Animal models

Male wild-type C57Bl/6 mice were generated in the KULeuven animal facility.

Mice were kept in individual microisolation cages on a 12 h day/night cycle and fed with a high-fat diet (HFD, Harlan Teklad TD88137, Zeist, the Netherlands; 42% kcal as fat, caloric value 20.1 kJ/g). Water was always available *ad libitum*. Body weight and food intake were measured daily, and body temperature was measured at weekly intervals using a rectal probe (TR-100, Fine Science Tools, Foster City, CA). Physical activity at night was monitored in cages equipped with a turning wheel linked to a computer to register full turns/12 h.

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At the end of the experiments, mice were anesthetized by intraperitoneal injection of 60 mg/kg Nembutal (Abbott Laboratories, North Chicago, IL). Blood was collected via the retroorbital sinus on trisodium citrate (final concentration 0.01 mol/l) and plasma was stored at −80 °C. Intra-abdominal (gonadal (GON)), inguinal subcutaneous (SC) or *de novo* formed fat pads were removed and weighed; portions were snap-frozen in liquid nitrogen for RNA extraction and paraffin sections (10 µm) were prepared for histology and immunohistochemistry. Other organs including kidneys, lungs, spleen, pancreas, liver, heart, and testes were also removed and weighed.

All animal experiments were approved by the local ethical committee (KULeuven P03112) and performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1996) and the guiding principles of the International Society on Thrombosis and Hemostasis (17). Fumagillin was obtained from AG Scientific (San Diego, CA; product no. F-1028).

Effect of fumagillin on *in vivo* adipose tissue formation

Effect on evolution of nutritionally induced obesity. Five-week-old male wild-type C57Bl/6 mice were kept on HFD for 6 weeks. At the age of 11 weeks, group 1 (n = 10) was started on fumagillin at a dose of 1 mg/kg/day in 0.25% dimethyl sulfoxide (DMSO) by oral gavage. Food intake was monitored, and 1 day later group 2 (n = 10) was started with the same volume of 0.25% DMSO given by oral gavage, whereas food was restricted to the mean amount consumed by group 1 (pair-feeding). Another day later, group 3 (n = 10) was started on 0.25% DMSO by oral gavage and *ad libitum* access to HFD. Citrated blood samples were taken at the start and before killing, after overnight fasting.

The study was continued for 4 weeks and monitored as described below.

Effect on established obesity. Five-week-old male wild-type C57Bl/6 mice were kept on HFD for 15 weeks to establish obesity. All mice were primed with 1% DMSO by oral gavage during 7 days while kept on HFD and their food intake was monitored individually on a daily basis. Then, one group received fumagillin at 1 mg/kg/day in 1% DMSO via oral gavage and had *ad libitum* access to the HFD. A control group (started 1 day later) was continued on 1% DMSO and restricted in HFD (on the basis of their food intake during the priming period and our previous experience with fumagillin), aiming at achieving a weight loss identical to that in the fumagillin treated group. The studies were continued for 2, 4, or 10 days (separately) and monitored as described below.

Analysis

The size and density of adipocytes or blood vessels in the adipose tissues were determined by staining with hematoxylin/eosin under standard conditions or with the Bandeiraea simplicifolia lectin, followed by signal amplification with the Tyramide Signal Amplification Cyanine System (Perkin Elmer, Boston, MA), as described (18,19). Blood vessel density was normalized to the adipocyte number. Analysis was performed using a Zeiss Axioplan 2 Imaging microscope with the AxioVision rel. 4.6 software (Carl Zeiss, Oberkochen, Germany).

Macrophage content of adipose tissues was quantitated following staining with anti-Mac-3 antibody (Pharmingen, San Diego, CA) as described above and expressed in percentage of the total section area. Collagen in adipose tissue sections was stained with Sirius red and quantified as percentage stained area per total tissue section area (20). Quality of collagen fibers was estimated by Sirius red polarization microscopy, allowing to quantify thick, tightly packed collagen fibers (orange-red) and thin, loosely assembled fibers (yellow-green) (21).

Blood glucose concentrations were measured using Glucocard strips (Menarini Diagnostics, Firenze, Italy). Other metabolic parameters and liver enzymes, including triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase were determined using standard laboratory assays. Insulin (Mercodia, Uppsala, Sweden) and leptin (R&D Systems Europe, Lille, France) levels were measured using commercial enzyme-linked immunosorbent assay and PAI-1 antigen with a specific home-made enzyme-linked immunosorbent assay (22).

Monitoring of mRNA expression

Adipose tissues were homogenized using lysing matrix tubes (Qbiogene, Carlsbad, CA) in a Hybaid ribolyser (Thermo, Wallham, MA). Total DNA-free RNA was extracted using the RNA Easy Qiagen kit (Qiagen, Valencia, CA) and RNA concentrations were determined with the RiboGreen RNA quantification kit (Molecular Probes, Eugene, OR). Samples were aliquoted and stored at −80 °C.

The expression of Met-AP2, Tie-2, Ang-1, and Ang-2 was determined by quantitative reverse transcription PCR, using specific primers and probes (Applied Biosystems, Foster City, CA). Reverse transcription reactions were performed from 50 ng of total RNA at 48 °C during 60 min with the TaqMan Reverse Transcription kit supplemented with 5 µmol/l random hexamers (Applied Biosystems). Quantitative real-time PCR was performed in the ABI 7500 fast sequence detector using the TaqMan Fast Universal PCR Master Mix (Applied Biosystems). As a housekeeping gene the expression of Glyceraldehyde 3-phosphate dehydrogenase was measured (Gene expression assay, Mm00441818_A1, Applied Biosystems). Analysis of the cycle threshold values of the real-time PCR was performed with the delta–delta cycle threshold method using the 7500 Fast System SDS software (Applied Biosystems).

Statistical analysis

Data are first averaged per mouse and are given as means ± s.e.m. for the number of animals studied. Statistical significance between groups is evaluated by nonparametric Mann–Whtiney U-test. Values of $P < 0.05$ are considered statistically significant.

Table 1 Effect of 4 weeks fumagillin treatment on adipose tissue and organ weights of mice with nutritionally induced obesity

	Group 1	Group 2	Group 3
Body weight start (g)	29.6 ± 0.39	29.2 ± 0.58	30.5 ± 0.85
Body weight day 28 (g)	25.6 ± 0.45	28.1 ± 0.39**	31.8 ± 0.69†
Body weight end[a] (g)	23.4 ± 0.46	26.5 ± 0.37***	29.6 ± 0.69†
SC fat (mg)	422 ± 28	560 ± 30**	926 ± 70†
GON fat (mg)	582 ± 54	777 ± 45*	1,314 ± 114†
Spleen (mg)	56 ± 2.1	68 ± 2.3**	78 ± 8.4*
Left kidney (mg)	155 ± 5.9	173 ± 6.1	179 ± 4.8**
Right kidney (mg)	164 ± 4.1	190 ± 6.2**	174 ± 3.7
Liver (mg)	1,085 ± 18	1,093 ± 37	1,360 ± 89**
Lung (mg)	156 ± 7.1	181 ± 12	153 ± 6.8
Pancreas (mg)	212 ± 7.2	271 ± 12**	277 ± 13†
Heart (mg)	121 ± 3.5	134 ± 3.2*	123 ± 1.8
Right testes (mg)	83 ± 7.5	79 ± 1.9	81 ± 3.6
Left testes (mg)	75 ± 1.9	80 ± 1.7	82 ± 3.1

Group 1, fumagillin at 1 mg/kg in 0.25% DMSO, HFD *ad libitum*; group 2, 0.25% DMSO, pair-feeding; group 3, 0.25% DMSO, HFD *ad libitum*.
[a] Body weight after overnight fasting (day 29).
Data are mean ± s.e.m. of 9 or 10 experiments: *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$, and †$P < 0.0001$ vs. group 1.

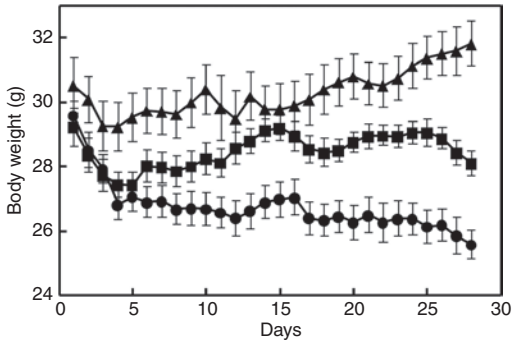

Figure 1 Evolution of body weight for mice treated with fumagillin at 1 mg/kg/day (circles, group 1), for control pair-fed mice treated with vehicle (squares, group 2), and for control mice fed HFD *ad libitum* (triangles, group 3).

RESULTS

Effect of fumagillin treatment on evolution of nutritionally induced obesity

To evaluate the effect of fumagillin treatment on ongoing development of obesity, 11-week-old mice (30 ± 0.39 g; n = 9)—previously kept on HFD for 6 weeks—were given fumagillin at a dose of 1 mg/kg/day during 4 weeks on HFD (group 1). Control mice (29 ± 0.58 g; n = 10) were pair-fed (HFD restricted to 2.8 ± 0.04 g/day, as compared to 2.8 ± 0.09 g/day consumed by group 1) and received vehicle (group 2). In addition, control mice (30 ± 0.85 g; n = 9) also received vehicle, but had *ad libitum* access to the HFD (group 3).

At day 28, the last day of HFD feeding, the body weight of fumagillin treated mice (group 1) was significantly lower than that of pair-fed mice (group 2) and of mice fed *ad libitum* (group 3) (**Table 1** and **Figure 1**). This corresponds to a significant weight loss in group 1 (P < 0.0001 at day 28 vs. start), but not in group 2 (P = 0.19) or group 3 (slight weight gain; P = 0.30). The SC and GON fat mass was significantly lower in group 1 as compared to group 2. The weight of some other organs, including spleen, kidney, pancreas, and heart was also lower in group 1. With HFD feeding *ad libitum* (group 3), SC and GON fat mass were markedly higher, as well as kidney, liver, and pancreas weight (**Table 1**). Body temperature remained constant at around 36 °C over the 4 week experimental period and was not different among the three groups (data not shown).

Physical activity at night was not significantly different in group 1 as compared to group 2 (3,300 ± 700 turns/night vs. 7,400 ± 1,900 turns/night (P = 0.09)).

Plasma metabolic parameters and liver enzymes at start and end of the experiment are summarized in **Table 2**. Insulin levels at the end were lower in group 2 as compared to groups 1 and 3. Leptin levels decreased during the experiment in groups 1 and 2, but not in group 3, resulting in significantly higher levels at the end. Total and high-density lipoprotein cholesterol as well as triglyceride levels at the end were elevated in group 3. Alkaline phosphatase levels at the end were significantly lower in group 1 as compared to groups 2 and 3.

Analysis of blood cell composition at the end of the experiment indicated significantly enhanced levels of neutrophils (45 ± 6.5% vs. 22 ± 3.6%; P < 0.005) and reduced levels of lymphocytes (51 ± 6.5% vs. 74 ± 4.4%; P < 0.05) in group 1 as compared to group 2. Total white blood cell counts were, however, not different (1.8 ± 0.35 × 10^3/μl vs. 2.4 ± 0.38 × 10^3/μl; P = 0.24). Other blood cell fractions were not significantly affected by fumagillin treatment (group 1 vs. 2). Furthermore, no significant differences were observed in blood cell analysis between groups 1 and 3 (data not shown).

Histological analysis revealed smaller adipocyte size in SC and GON adipose tissues of fumagillin treated mice, as

Table 2 Effect of 4 weeks fumagillin treatment on plasma metabolic parameters and liver enzymes of mice with nutritionally induced obesity

	Group 1	Group 2	Group 3
Glucose (mg/dl) end	89 ± 9.3	88 ± 10	107 ± 10
Insulin (ng/ml)			
Start	1.8 ± 0.55	1.9 ± 0.55	1.9 ± 0.30
End	1.6 ± 0.25	0.82 ± 0.12*	1.6 ± 0.31
Leptin (ng/ml)			
Start	6.3 ± 0.95	7.3 ± 1.1	10 ± 1.6
End	1.5 ± 0.22	1.7 ± 0.33	12 ± 1.7***
PAI-1 (ng/ml)			
Start	5.2 ± 0.58	3.9 ± 0.29	5.7 ± 0.56
End	6.1 ± 1.5	4.5 ± 0.26	6.6 ± 0.58
Total cholesterol (mg/dl)			
Start	165 ± 6	172 ± 8	182 ± 15
End	101 ± 4	98 ± 9	139 ± 12*
HDL cholesterol (mg/dl)			
Start	155 ± 6	151 ± 7	157 ± 15
End	95 ± 5	92 ± 8	127 ± 11*
LDL cholesterol (mg/dl)			
Start	26 ± 1	31 ± 2	38 ± 3
End	18 ± 2	20 ± 2	20 ± 2
Triglycerides (mg/dl)			
Start	27 ± 1	39 ± 5	43 ± 2
End	22 ± 3	15 ± 1*	48 ± 3***
Alkaline phosphatase (U/l)			
Start	195 ± 22	149 ± 13	173 ± 18
End	75 ± 9	122 ± 3***	163 ± 27***
AST (U/l)			
Start	108 ± 12	54 ± 3	98 ± 31
End	100 ± 5	90 ± 4	118 ± 26
ALT (U/l)			
Start	80 ± 11	40 ± 8	59 ± 20
End	44 ± 2	48 ± 6	71 ± 10*

Data are mean ± s.e.m. of 6–10 determinations.
*P < 0.05, **P < 0.005, and ***P < 0.0005 (only calculated for end values) vs. group 1.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; PAI-1, plasminogen activator inhibitor-1.

compared to both control groups, associated with higher adipocyte density. Overall, fumagillin treatment induced marked adipocyte hypotrophy associated with enhanced density as compared to mice fed HFD *ad libitum* (Figure 2), but less marked as compared to pair-fed control mice (Table 3).

Quantitative analysis of blood vessel size and density did not show an effect of fumagillin treatment either in SC or GON adipose tissues, as compared to pair-fed controls (except for a larger blood vessel size in GON fat). Mice fed the HFD *ad libitum* had significantly lower blood vessel size and density (Table 3). The number of blood vessels surrounding each adipocyte (normalized blood vessel density) thus is lowest in the fumagillin treated group, and is for the GON fat significantly lower than for pair-fed or *ad libitum* fed controls.

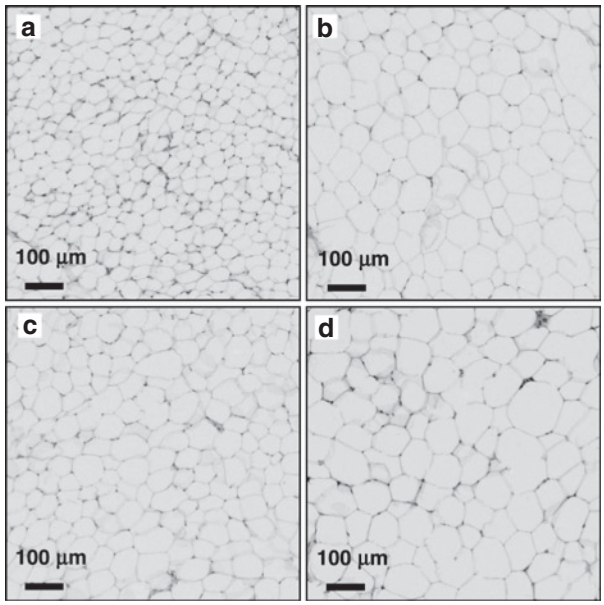

Figure 2 Hematoxylin-Eosin staining of subcutaneous (**a** and **b**) and gonadal (**c** and **d**) adipose tissue sections of mice treated with fumagillin at 1 mg/kg/day (group 1) (**a** and **c**) or controls with DMSO fed HFD *ad libitum* (group 3) (**b** and **d**).

Table 3 Effect of 4 weeks fumagillin treatment on adipocyte and blood vessel size and density in adipose tissues of mice with nutritionally induced obesity

	Group 1	Group 2	Group 3
Adipocyte size (µm²)			
SC fat	2,006 ± 292	2,453 ± 206	3,856 ± 313***
GON fat	3,097 ± 143	3,697 ± 119***	4,757 ± 303†
Adipocyte density (×10⁻⁶/µm²)			
SC fat	588 ± 72	468 ± 40	292 ± 26***
GON fat	322 ± 15	277 ± 8**	217 ± 14†
Blood vessel size (µm²)			
SC fat	65 ± 3.7	57 ± 2.1	48 ± 2.9***
GON fat	89 ± 6.7	69 ± 5.3*	60 ± 4.2**
Blood vessel density (×10⁻⁶/µm²)			
SC fat	524 ± 36	524 ± 23	358 ± 25***
GON fat	323 ± 16	317 ± 16	261 ± 20*
Normalized blood vessel density			
SC fat	0.96 ± 0.08	1.16 ± 0.06	1.27 ± 0.10
GON fat	0.98 ± 0.04	1.15 ± 0.04*	1.22 ± 0.10*

Data are mean ± s.e.m.
SC, subcutaneous; GON, gonadal.
*P < 0.05, **P < 0.01, ***P < 0.005, and †P < 0.0005 vs. group 1.

Reverse transcription PCR on extracts of SC and GON adipose tissues did not reveal an effect of fumagillin treatment on MetAP2 mRNA expression (group 1 vs. group 2). Normalized ΔCt values were 6.95 ± 0.14 vs. 7.28 ± 0.19 for SC fat (1.3-fold upregulation; P = 0.32), with corresponding values of 6.99 ± 0.15 vs. 7.34 ± 0.19 for GON fat (1.3-fold upregulation; P = 0.09).

Effect of short-term fumagillin treatment on established obesity

To evaluate the effect of short-term fumagillin treatment on obesity, 20-week-old obese mice (43 ± 1.1 g; n = 10) were treated at a dose of 1 mg/kg/day during 4 days and a control group (43 ± 1.3 g; n = 8) received solvent. Body weight and food intake (HFD) of the fumagillin group were monitored daily and the food allocated to the control mice was adjusted to match the body weight of the treated group. With a food intake of 2.2 ± 0.05 g/day for the controls, their body weight after 4 days matched that of the fumagillin group (40 ± 1.4 g vs. 40 ± 1.2 g) that consumed 2.6 ± 0.08 g HFD/day (P = 0.004 vs. control). The weights of the isolated SC and GON fat pads were also very similar in both groups (Table 4). The weight of other organs, including spleen, kidney, liver, lung, pancreas,

Table 4 Effect of 4 day fumagillin treatment of obese mice on adipose tissue weight and composition

	Fumagillin (n = 10)	Control (n = 8)
Body weight start (g)	43 ± 1.1	43 ± 1.3
Body weight end (g)	40 ± 1.2	40 ± 1.4
Food intake (g/day)	2.6 ± 0.08	2.2 ± 0.05**
Weight SC fat (mg)	1,330 ± 65	1,380 ± 86
Weight GON fat (mg)	2,000 ± 94	2,110 ± 60
Adipocyte size (µm²)		
SC fat	2,900 ± 120	3,488 ± 150*
GON fat	5,130 ± 240	5,740 ± 130
Adipocyte density (×10⁻⁶/µm²)		
SC fat	360 ± 16	300 ± 14***
GON fat	200 ± 9.4	180 ± 3.8
Blood vessel size (µm²)		
SC fat	34 ± 1.5	32 ± 1.3
GON fat	56 ± 4.0	66 ± 8.3
Blood vessel density (×10⁻⁶/µm²)		
SC fat	270 ± 23	220 ± 24
GON fat	220 ± 11	210 ± 13

Data are mean ± s.e.m. of n experiments.
SC, subcutaneous; GON, gonadal.
*P < 0.05, **P < 0.005, and ***P 0.0005 vs. fumagillin.

and heart was also indistinguishable in both groups (data not shown). Thus, despite a significantly higher food intake, a similar weight loss was observed in fumagillin treated mice as compared to control mice receiving 1% DMSO (3.0 ± 0.22 g vs. 2.9 ± 0.21 g). This was not associated with effects on metabolic parameters: plasma levels of leptin, insulin, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase (measured at the start and while killing) were not significantly different in both groups (data not shown). In addition, blood cell analysis at the end of the experiment did not reveal differences between the fumagillin and vehicle groups (data not shown).

Histological analysis of SC fat pads revealed a significantly smaller adipocyte size ($P = 0.012$) and higher density ($P = 0.0002$) in fumagillin treated samples as compared to controls. A similar trend, but not statistically significant, was observed in GON adipose tissues. Analysis of blood vessel size and density did not reveal significant effects of fumagillin treatment as compared to vehicle (**Table 4**).

Short-term treatment with fumagillin had no effect on the macrophage content of SC fat (0.86 ± 0.06% vs. 0.70 ± 0.03% in controls) or of GON fat (0.74 ± 0.06% vs. 0.78 ± 0.10% in controls). The collagen content was similar for fumagillin and vehicle treated SC adipose tissues (3.9 ± 0.29% vs. 3.8 ± 0.32%) as well as GON adipose tissues (1.8 ± 0.17% vs. 1.8 ± 0.24%). Also the collagen organization (monitored as ratio thick/thin fibers), was similar for SC fat (1.1 ± 0.09 vs. 1.3 ± 0.26 in controls) and for GON fat (0.52 ± 0.11 vs. 0.41 ± 0.10 in controls).

Monitoring mRNA expression levels revealed upregulation by fumagillin treatment in GON adipose tissues of Met-AP2 (3.5-fold as compared to control; $P < 0.005$), TIE-2 (2.7-fold; $P < 0.005$), Ang-1 (1.9-fold; $P < 0.05$) and Ang-2 (3.5-fold; $P < 0.0005$). In contrast, no significant effects on these targets were observed in SC adipose tissues (1.1- to 1.3-fold upregulation).

Similar observations were made using the same experimental protocol with 2 or 10 day fumagillin treatment. In both studies, comparable weight reduction was achieved by food adjustment in fumagillin and vehicle treated groups.

Fumagillin treatment for 2 days resulted in a weight loss of 1.6 ± 0.43 g ($n = 6$), as compared to 1.5 ± 0.44 g ($n = 6$) in the control group. Average food intake during the first 24 h was 3.6 ± 0.20 g in the fumagillin group and 3.9 ± 0.24 g in the control group. During the second 24 h period, food in the control group was restricted to 1.8 ± 0.31 g, as compared to 2.0 ± 0.24 g in the fumagillin group.

Fumagillin treatment for 10 days resulted in a weight loss of 3.7 ± 0.35 g ($n = 8$), as compared to 3.4 ± 0.45 g ($n = 7$; $P = 0.96$) in the control group. Average food intake over 10 days was 2.9 ± 0.11 g/day for the treated group and 2.5 ± 0.07 g/day ($P = 0.03$) for the control group. Both after 2 and 10 day treatment, body weight as well as SC and GON fat mass, were comparable for treated and control groups. No significant effect of fumagillin treatment was observed on blood vessel size or (normalized) density in SC or GON fat (data not shown).

DISCUSSION

The expansion of adipose tissue is linked to the development of vasculature, as adipogenesis is tightly associated with angiogenesis (3,4). *In vitro* studies indeed revealed that adipose tissue explants in fibrin or collagen gels trigger blood vessel formation (23) and that in turn adipose tissue endothelial cell promote preadipocyte differentiation (24). Furthermore, it was shown that adipocytes and endothelial cells are derived from a common progenitor cell (25). A recent study demonstrated the *in vivo* formation of a robust functional vascular network by cooperation of adipose progenitor (stromal) and endothelial progenitor cells (26). Adipose tissue produces and secretes many different types of pro- and antiangiogenic factors (5). Several *in vivo* studies have suggested that inhibition of the development of the vascular network in adipose tissue may constitute a strategy to affect obesity (5–9).

It was shown that adipose tissue growth in mice can be impaired with angiogenesis inhibitors such as TNP-470, a synthetic analog of fumagillin that selectively inhibits endothelial cell growth by suppression of methionine aminopeptidase (6). A comparative study of the antiangiogenic properties of fumagillin and TNP-470 (AGM-1470) concluded that the TNP-470 derivative was superior in terms of potency, selectivity for endothelial cells and toxicity (27). In this study, we have re-investigated the effect of the parent molecule, fumagillin in murine models of obesity. We have used a daily dose of 1 mg/kg on the basis of preliminary experiments in mice. Overall, fumagillin was well tolerated without apparent toxic side effects. With mice kept on HFD and pair-fed, overall a reduction of body weight and of SC and GON fat mass was observed, associated with adipocyte hypotrophy. We did not observe differences in collagen content or in macrophage infiltration in fumagillin treated adipose tissues. Interestingly, we did not observe a good correlation between adipose tissue mass and plasma PAI-1 levels, as was previously reported for mice and man (28,29). This may be due to the fact that PAI-1 levels are already significantly elevated in all 3 groups (at the start), as a result of the 6 weeks HFD feeding. Indeed, plasma PAI-1 levels in mice are normally in the range 1–2 ng/ml (30).

Our experiments in mice with established obesity and short-term treatment indicate that, despite a significantly higher food intake of fumagillin treated mice as compared to controls, a similar weight loss was observed without marked effects on metabolic parameters. This may suggest that energy expenditure is higher in fumagillin treated mice, although we did not observe differences in body temperature or physical activity. Also in a previous study with TNP-470 (8) in mice on HFD, no effect on energy expenditure was observed.

In these experiments, short-term administration of fumagillin was performed under feeding conditions monitored to keep the body weight of treated and control mice comparable. Because also fat mass remained comparable in both groups,

these experiments allow to exclude an effect of reduced total fat mass on angiogenesis. Under these conditions, we did not observe an effect of fumagillin on blood vessel size or density in fat tissues. If blood vessel density was normalized to the adipocyte number (because the number and/or size of adipocytes may affect blood vessel density (31)) a somewhat lower normalized density was observed in SC and GON fat following fumagillin treatment of mice developing obesity (4 weeks treatment). These findings suggest that analysis of blood vessel size/density in developing adipose tissue has to be interpreted with care, because it depends on total fat mass, and adipocyte size/density.

Thus, our data confirm that treatment with fumagillin significantly impairs diet-induced obesity in mice; the mechanism may involve induction of adipocyte hypotrophy, without, however, a marked effect on adipose tissue angiogenesis.

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DISCLOSURE

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